Establishment of plant regeneration system for young leaf explants of *Cassia mimosoides*

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Abstract. In the present study, a plant regeneration system was established by using young leaf explants from aseptic seedlings of *Cassia mimosoides*. The results showed that when the explants were inoculated on the 1/2MS medium contained 2.0 mg/L 6-BA and 0.5 mg/L NAA, high rate of callus induction and good growth status of adventitious bud, and the highest number of buds were obtained. Further studies conclusively suggested that IBA could not promote root formation effectively, but the root regeneration could be induced by NAA. However, although the rooting of regeneration buds could be induced when adventitious buds were placed into 1/2MS medium supplemented with 0.5 mg/L NAA, the rate of rooting was reduced, and the roots became shorter. Therefore, the best medium for adventitious bud regeneration using young leaf as explants in *C. mimosoides* was 1/2MS medium contained 2.0 mg/L 6-BA and 0.5 mg/L NAA, and the best rooting medium was 1/2MS.

1 Introduction

Cassia mimosoides Linn., belongs to the genus of *Cassia* in the legume family [1]. It is mainly distributed in the provinces of Guangdong, Guangxi, Sichuan, Hunan and Fujian in China. It has the effect of clearing away heat and detoxifying, invigorating spleen and dampness, and purging, mainly used for treating many diseases such as jaundice, vomiting and diarrhea, infantile malnutrition and edema, habitual constipation and malignant boils and so on [2]. The chemical components of *C. mimosoides* include anthraquinones, flavonoids, sterols, etc. [3]. Domestic and foreign studies on the medicinal activity of *C. mimosoides* are mainly focused on its hepatoprotective effect. Some studies suggest that the compounds of anthraquinone in *C. mimosoides* have strong inhibitory effect on lipase, which can prevent and improve body obesity, liver hypertrophy and hypertriglyceridemia caused by high fat diet [4, 5]. Furthermore, a series of studies have found that the active substances extracted from *C. mimosoides* can protect liver tissue by lowering lipid, inhibiting liver fibrosis and enhancing anti-oxidation [6, 7, 8]. The serum containing the active components of *C. mimosoides* has a protective effect on liver cell injury [9].

Because of its high medicinal value, *C. mimosoides* is in great demand on the market. However, due to the low seed germination rate, long growth cycle, lack of high-quality germplasm resources and other problems in the cultivation process, the yield and quality of *C.*

mimosoides are difficult to meet the increasing demand of the market. Therefore, this study intends to establish a tissue culture regeneration system for shortening the growth cycle and increasing the yield of *C. mimosoides*, which will provide the early foundation for expanding the planting area of *C. mimosoides*. It also provides raw material for the further analysis of biochemical activities and lays a foundation for applied basic research of liver protection.

2 Materials and Methods

2.1 Plant materials

In this study, the seeds of *C. mimosoides* were collected from Yinna Mountain (at 24 degrees north and 116 degrees longitude), Meizhou City, Guangdong Province in China.

2.2 Experimental reagents

The main reagents used in this study were 6-BA (6-benzylamino purine), IBA (indolebutyric acid), NAA (naphthalene acetic acid), sucrose, inositol and agar powder, which were purchased from Beijing Solarbio Science & Technology Co., Ltd.

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2.3 Culture conditions

1/2MS medium [10] supplemented with 30 g/L sucrose and 8 g/L agar was served as the basic medium. The pH value of the medium was adjusted to 5.8, and the medium was placed in autoclave under conditions of 121°C and 0.1 MPa for sterilization for 20 min. The culture conditions of the incubator were set to adjust light at 12 h, light intensity at 2000~2500 lx, humidity at 80%, and temperature at 22°C.

2.4 Experimental methods

2.4.1 Germination of seeds

The *C. mimosoides* seeds were soaked in water at 80°C for 5 minutes, washed firstly with 70% ethanol for 2-3 times and then with 5% NaClO for 5-10 minutes, finally with sterile distilled water for 3 times. The sterilized seeds were inoculated on 1/2MS medium for 8 days of culture. Young leaves (size with 0.5 cm × 0.5 cm squares) were taken from the asepsis seedlings as explants.

2.4.2 Callus and adventitious bud induction

The young leaf explants were inoculated horizontally on 1/2MS medium contained 2.0 mg/L 6-BA and different concentrations of NAA (0, 0.5 and 1 mg/L) for callus and adventitious bud induction for 80 days of culture.

2.4.3 Screening of rooting medium

The regeneration buds (longer than 1 cm) were vertically inoculated on 1/2MS medium supplemented with different concentrations of IBA (0, 0.5 and 1.0 mg/L) and NAA (0, 0.1, 0.2 and 0.5 mg/L) respectively for rooting for 20 days.

2.4.4 Data analysis

The induction rate of callus = (number of explants with callus/total number of inoculated explants) × 100%; The induction rate of adventitious bud = (number of explants with buds/total number of inoculated explants) × 100%; Rooting coefficient = (number of roots/number of root explants) × 100%; Rooting rate = (number of rooting shoots/number of inoculated shoots) × 100%. The data was analysed by the software SPSS 22.0 and conducted with Duncan multiple comparisons ($P \leq 0.05$). And significant difference was represented by different letters.

3 Results

3.1 Effects of NAA on the induction of callus and adventitious bud

For the purpose of studying the effect of NAA on callus formation and adventitious bud induction from the

young leaf explants, which were inoculated on basal medium supplemented with different concentrations of NAA for 80 days of culture. The results showed that the maximum callus induction rate (98.5%) and adventitious shoot regeneration rate (60%) were gained when the concentration of NAA was at 0.5 mg/L, while the regeneration effect would be restrained with continuing to increase NAA concentration. These results suggested that NAA had positive effect on improving callus formation and adventitious bud regeneration, and the optimal concentration of NAA was 0.5 mg/L.

Table 1. Results of NAA on the induction of callus and adventitious bud

Concentrations of NAA (mg/L)	Callus induction rate (%)	Induction rate of adventitious bud (%)
0	81.1±5.1b	33.3±6.7b
0.5	98.5±2.0a	60.0±11.5a
1	93.7±7.5ab	6.67±0.1c

Note: Different letters after the data indicated significant differences.

Figure 1. Effects of NAA on the induction of callus and adventitious bud in *C. mimosoides*

Young leaf explants were inoculated on 1/2MS medium contained 2.0 mg/L 6-BA and A: 0 mg/L, B: 0.5 mg/L, C: 1 mg/L NAA for induction of callus and adventitious bud in *C. mimosoides* for 80 days. (Bars=1 cm)

3.2 Influences of IBA and NAA on rooting of regeneration shoots

In order to investigate the effect of IBA and NAA on the rooting effect of the regeneration shoots, which were placed into 1/2MS medium contained various concentrations of IBA and NAA for 20 days of culture. The results indicated that IBA could not effectively promote the rooting of *C. mimosoides* regeneration shoots, and with the increasing of IBA concentration, the rooting efficiency of shoots decreased gradually (Table 3). The study also found that NAA had a certain effect on the rooting of shoots. Although the number of roots increased, the rooting rate would gradually reduce with the increasing of NAA concentration (Table 4, Figure 2). Therefore, 1/2MS medium without IBA or NAA was the most suitable medium for rooting induction.

Table 2. Results of different concentration of IBA on rooting of regeneration shoots

Concentrations of IBA (mg/L)	Rooting coefficient	Rooting rate (%)
0	5.2±0.4a	96.7±5.0a
0.5	2.8±1.6b	80±8.7b
1	0.8±1.0b	16.7±3.9c

Note: Different letters after the data indicated significant differences.

Table 3. Effects of various concentration of NAA on rooting of regeneration shoots

Concentrations of NAA (mg/L)	Rooting coefficient	Rooting rate (%)
0	3.3±0.8b	93.8±2.4a
0.1	4.0±1.6ab	86.1±8.3ab
0.2	9.0±1.0a	79.2±5.1b
0.5	11.0±1.0a	65.6±6.9b

Note: Different letters after the data indicated significant differences.

Figure 2. Effects of NAA on rooting of regeneration shoots in *C. mimosoides*

Regenerated shoots were inoculated on 1/2MS medium supplemented with A: 0 mg/L, B: 0.1 mg/L, C: 0.2 mg/L, D: 0.5 mg/L NAA for rooting in *C. mimosoides* for 20 days. (Bars=1 cm)

4 Discussion

4.1 Effects of hormones on the induction of callus and adventitious bud

Plant hormones are a series of extremely low levels of naturally occurring organic compounds in plants. The interactions among various plant hormone signals regulate the growth and differentiation of plant cells. Plant hormones play an important role in the normal development of plants. NAA is a member of auxin family, while 6-BA is one of the cytokinin species, both of them play an important part in induction of callus formation and adventitious bud regeneration. Our results revealed that both NAA and 6-BA were effective in

inducing callus and adventitious bud regeneration. Some studies show that the signaling pathways between auxin and cytokinin influence callus formation and adventitious bud regeneration through different contributing factors [11].

4.2 Effects of IBA and NAA on rooting of regeneration shoots

Root is important organs for plant to absorb water and nutrients, which can provide various substances for plant growth and development. It is very important for the establishment of plant tissue culture regeneration system to investigate whether regeneration buds can take root. Therefore, it is very necessary to explore the suitable nutrient content and hormone concentration in the medium for the rooting of regeneration shoots. In this study, it was found that regeneration shoots had the best effect of rooting in 1/2MS medium, while the growth of root was inhibited by the addition of hormones. For example, the induction of adventitious root was restrained after adding different concentrations of IBA in medium. Meanwhile, adding different concentrations of NAA could promote the increasing of number of roots, but the roots became shorter and bulkier, and some callus even grown on the roots and the rooting rate decreased. These results are very similar to some that have been reported [12]. Hence, 1/2MS medium was the most suitable medium for the rooting of adventitious shoots of *C. mimosoides* in the present study.

Application of the established plant regeneration system from young leaf explants of *C. mimosoides* in this study, the incubation time of the plant could be reduced, and the yield of plant material might be increased. However, there are still the following problems in the development of *C. mimosoides* industry, which are worth further discussion. Firstly, the addition of exogenous hormones may cause changes in the ratio and level of endogenous hormones, so as to further regulate the growth of cultures. Although the effect of adding exogenous hormones in the tissue culture of *C. mimosoides* has been clarified in this study, the mechanism of action between various hormones is still unclear. Therefore, the investigation of the physiological mechanism of endogenous hormones in the tissue culture process of *C. mimosoides* will provide a theoretical basis for the use of exogenous hormone concentrations and different hormone combinations in the future, and also provide reference for the protection of medicinal plant resources of *Cassia* and industrial seedling cultivation. Secondly, the quality of tissue culture seedlings must be further improved to provide raw materials for the large-scale artificial planting of *C. mimosoides*. On the basis of tissue culture combined with modern biological breeding technology, excellent varieties with good quality and strong disease resistance will be cultivated, and scientific and standardized planting will be carried out to achieve greater economic benefits.

5 Conclusion

In this study, a method for plant regeneration of young leaf explants from aseptic seedlings of *Cassia mimosoides* was established successfully. Our preliminary results suggested that when the explants were placed onto the 1/2MS medium supplemented with 2.0 mg/L 6-BA and 0.5 mg/L NAA, high callus induction rate and good growth status of adventitious bud, and the highest number of buds were gained. Further research results revealed that IBA could not promote root formation effectively, but the roots could be induced by NAA. However, although the rooting of buds could be induced when adventitious shoots were inoculated into 1/2MS medium contained 0.5 mg/L NAA, only poor rooting effect could be obtained. In conclusion, the best medium for induction of adventitious bud regeneration using young leaf as explants in *C. mimosoides* was 1/2MS medium supplemented with 2.0 mg/L 6-BA and 0.5 mg/L NAA, and the optimum rooting medium was 1/2MS.

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